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DESIGN OF A QUANTITATIVE DEPT NMR EXPERIMENT For ^{13}C ACQUISITIONS

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14. ABSTRACT
A scheme has been developed to eliminate virtually all signal intensity dependence on $^{1}J_{CH}$ in polarization transfers between ^{1}H and ^{13}C nuclei, reducing the differences in signal intensity to only 1.5% over the entire natural $^{1}J_{CH}$ range. The scheme relies on the summation of time-domain data acquired with four suitably selected Δ delays so that the J dependence is essentially cancelled in the final signal averaged free induction decay. These Δ delays have been incorporated into the DEPT pulse sequence to create sensitivity-enhanced experiments for collecting quantitative $^{13}C\{^{1}H\}$ spectra. Four experiments, each with unique read pulse angles, give quantitative spectra with 200-300% more sensitivity than conventional ^{13}C spectra acquired with inverse-gated ^{1}H decoupling. The experiments are ideal for recording spectra with improved quantitative information or for substantially reducing the long acquisition times indicative of quantitative ^{13}C experiments. The ability of the experiments to provide quantitative spectra was demonstrated with a simple ethylbenzene solution; however, they can easily be adapted to various applications for analysis of complex mixtures.

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PREFACE

The work described in this report was started in July 2010 and completed in February 2011.

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1. INTRODUCTION

Since the pioneering work of Schoolery and Smithson [1] over 40 years ago, quantitative nuclear magnetic resonance (NMR) spectroscopy has taken a prominent role in chemical analysis. When acquired under conditions allowing complete signal relaxation, ^{1}H spectra provide quantitative information for small molecules or simple mixtures of low molecular weight compounds. However, as ^{1}H line widths increase with molecular weight, spectral signals often overlap, and signals of major components can obscure those of target analytes at low concentrations. In these cases, deconvolution or single-value decomposition methods are required for quantitative analysis. A much simpler and typically preferred strategy is to use ^{13}C spectroscopy, as the broader range for ^{13}C chemical shifts affords much higher spectral resolution. Unfortunately, the low sensitivity of the ^{13}C nuclei and long relaxation delays necessary at extreme narrowing conditions typically make obtaining adequate signal-to-noise ratios time consuming.

Outside of simple means such as optimizing experimental temperature, exploiting two different nuclear magnetic phenomena can be used to significantly enhance ^{13}C sensitivity. The $^{13}C\{^{1}H\}$ nuclear Overhauser effect (NOE), a manifestation of ^{13}C-^{1}H dipolar relaxation, can enhance ^{13}C sensitivity by as much as 299% [2]. Because cross-relaxation is intimately related to molecular dynamics, NOE enhancements can vary dramatically from one molecule to the next. Moreover, enhancements are difficult to predict and cannot be controlled adequately for use in quantitative spectroscopy. Polarization transfer on the other hand, requiring scalar coupling between ^{1}H and ^{13}C nuclei ($^{1}J_{CH}$ coupling) in this case, can increase ^{13}C sensitivity by as much as γ_{1H}/γ_{13C} or 398%. For pulse sequences incorporating polarization transfer steps, such as insensitive nuclei enhancement by polarization transfer (INEPT), distortionless enhancement by polarization transfer (DEPT), and heteronuclear single quantum correlation (HSQC), delay periods are used to transfer magnetization between ^{1}H and ^{13}C nuclei. Invariably, just one delay period (commonly designated as Δ) optimized for a single $^{1}J_{CH}$ coupling is used; this is typically 145 Hz. Under these conditions, ^{13}C signal intensity is strongly dependent on $^{1}J_{CH}$ (Figure 1), destroying any utility for polarization transfer in quantitative analysis. Reported herein is a scheme devised to cancel the signal intensity dependence on $^{1}J_{CH}$ in polarization transfers between ^{1}H and ^{13}C nuclei. The scheme has been incorporated into the DEPT pulse sequence to provide sensitivity-enhanced, quantitative DEPT (Q-DEPT) experiments.

2. THEORETICAL COMPUTATIONS AND EXPERIMENTAL DESIGN

The signal intensity J dependence of polarization transfers between ^{1}H and ^{13}C nuclei (described by eq 1 [3] and illustrated in Figure 1), optimized for $^{1}J_{CH}$ = 145 Hz, can result in signal intensity (I) differences of up to ca. 240%.

$$I \propto \sin^{2}(\pi\Delta^{1}J_{CH}) \qquad (1)$$

The devised scheme is based on the summation of time domain data acquired with suitably selected Δ values so that all J dependence is virtually eliminated in the final, signal averaged, free induction decay. The Δ values were determined by iteratively minimizing the difference between maximum and minimum I over the natural $^1J_{CH}$ range (115-220 Hz), an approach used to design quantitative HSQC spectroscopy [3]. Iterations were constrained to all $\Delta < 6$ ms to reduce spin-spin relaxation losses and evolution of homonuclear couplings during polarization transfers. Four Δ values (2.67, 3.11, 3.12, and 5.96 ms) were found to average the span of signal intensity from ^1H-^{13}C polarization transfers to only 1.5% over the natural $^1J_{CH}$ range (Figure 1). Attempts using two values were not successful, and those using eight values gave no improvement (not shown).

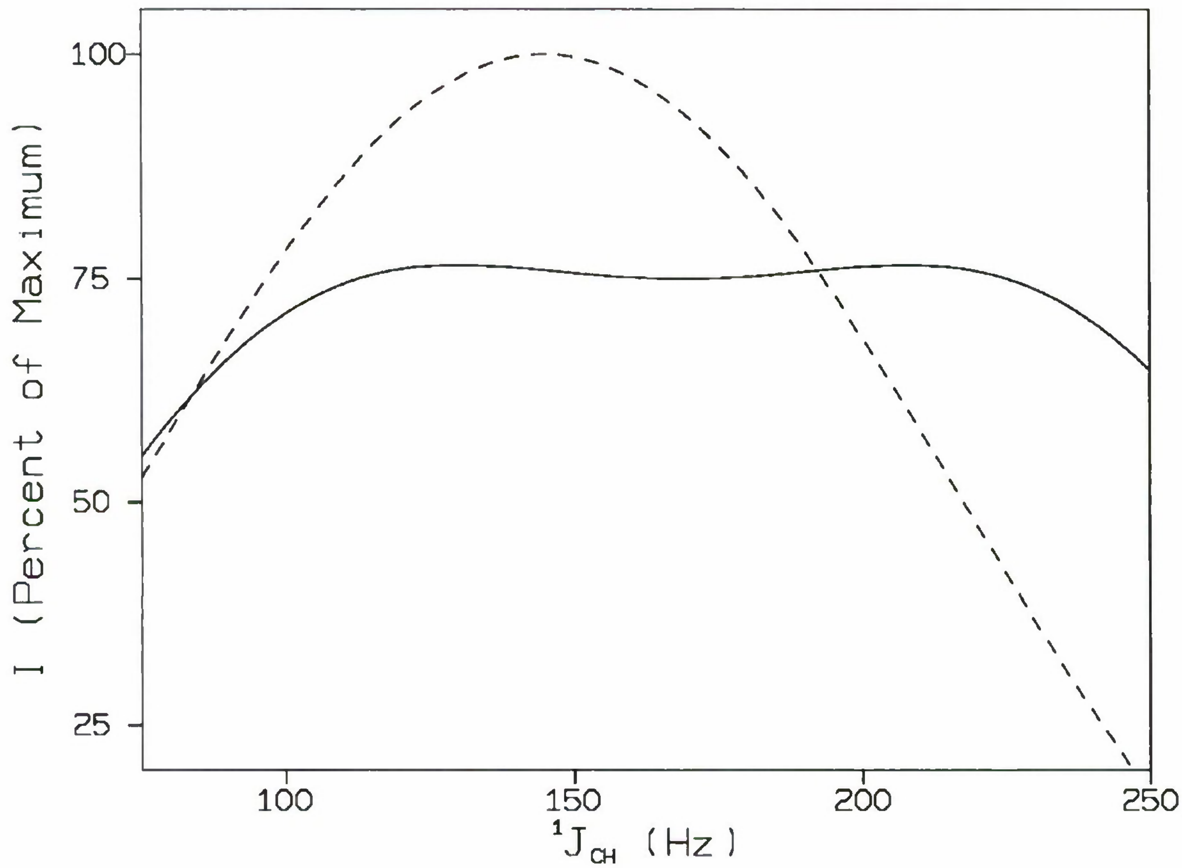

Figure 1. Simulated J Dependence of DEPT (broken line) and Q-DEPT (solid line) Spectroscopy.

Figure 2 shows the data-averaging scheme incorporated into the DEPT pulse sequence [4]. The angle θ of the read pulse generates both the intensity and positive or negative phase of all signals in the resulting spectrum. This dependency on θ is unique for ^{13}C nuclei directly bonded to one, two, or three ^{1}H nuclei, and is described by eqs 2, 3, and 4 [5], respectively

$$I_{CH} = (\gamma_{1H}/\gamma_{13C}) \sin \theta \tag{2}$$

$$I_{CH2} = (\gamma_{1H}/\gamma_{13C}) \sin 2\theta \tag{3}$$

$$I_{CH3} = [3\gamma_{1H}/4\gamma_{13C}] (\sin \theta + \sin 3\theta) \tag{4}$$

For quaternary carbon atoms, the absence of a directly bonded ^{1}H nucleus prevents polarization transfer and a corresponding signal in DEPT spectra. Any two of three equations can be solved simultaneously for θ to find a read pulse giving the same intensity and phase for their respective type of carbon atom. The third type will have an intensity and phase described by the remaining equation when solved for the derived θ. For example, eqs 2 and 3 can be solved simultaneously to find that a 60° read pulse gives the same signal intensity for methine and methylene ^{13}C nuclei. Equation 4 can then be solved for $\theta = 60°$ to find the relative intensity of methyl ^{13}C signals.

The results of solving the equations for all pairwise combinations are reported in Table 1 (experiments 1-3). The table also lists the Q-DEPT theoretical sensitivity enhancements for all protonated carbon atoms relative to conventional quantitative $^{13}C\{^{1}H\}$ spectroscopy [6]. A set of four additional θ values appearing in the table (experiment 4) is a series of read pulse angles that, when cycled together with the data-averaging scheme (see the legend for Figure 2), give spectra with uniform sensitivity enhancements for all detectable ^{13}C signals. These pulse angles were also found iteratively; eqs 2, 3, and 4 were solved simultaneously for four adjustable θ values until they converged on the same, pre-assigned value of signal intensity. This value was incremented and iteration continued. The entire process was repeated until convergence was no longer possible. Attempts using eight θ values gave only a marginal (ca. 1%) enhancement in sensitivity. All computation and mathematical modeling was conducted with the program MLAB (Civilized Software, Silver Spring, MD) on Windows XP (Microsoft Corp., Redmond, WA) personal computer platforms.

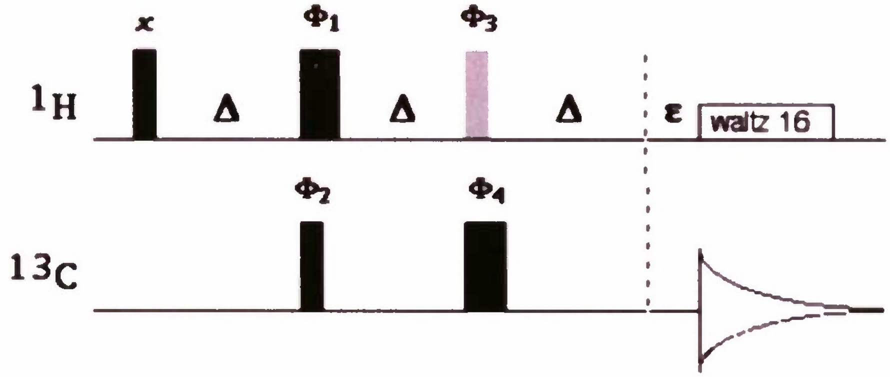

Figure 2. Q-DEPT Pulse Sequence. Thin and thick bars are 90 and 180° pulses, respectively, and the read pulse is the gray bar. The Δ delays are cycled 12(2.67 ms), 12(3.11 ms), 12(5.96 ms), 12(3.12 ms) so that each acquisition is recorded with three delays of the same value. The delay ε compensates for chemical shift evolution during pulses. The phase of the first pulse is held at x, while the other pulses and the receiver, are phase-cycles as follows: $\Phi_1 = x, -x, y, -y$; $\Phi_2 = 8(x), 8(-y), 8(-x), 8(-y)$; $\Phi_3 = 4(y), 4(-y)$; $\Phi_4 = 4(x, -x), 4(y, -y)$; $\Phi_{rcv} = 2(y), 4(-y), 2(y), 2(-x), 4(x), 2(-x), 2(-y), 4(y), 2(-y), 2(x), 4(-x), 2(x)$. For the uniform sensitivity enhancement experiment (Table 1, experiment 4), ϕ are cycled 4(50.0°), 4(35.6°), 4(49.1°), 4(84.9°). ^{1}H decoupling is by the WALTZ 16 sequence [7].

Table 1 reveals that Q-DEPT spectroscopy should be about 200-300% more sensitive than its conventional counterpart. Substantial reductions in acquisition times can still be realized using the quantitative experiments though Q-DEPT spectroscopy is 25% less sensitive than routine DEPT spectroscopy with Δ delays optimized for specific signals. For any one sample, Q-DEPT spectra will have similar signal-to-noise ratios as conventional quantitative ^{13}C spectra acquired 4-9 times longer under the same conditions.

Table 1. Read Pulse Angles and Sensitivity Enhancements for Q-DEPT Spectroscopy

		Sensitivity Enhancement (%)[c]		
Experiment	ϕ (deg)[a,b]	CH	CH_2	CH_3
1	45.0	213	301	301
2	54.8	246	284	246
3	60.0	261	261	196
4	$(50.0+35.6+49.1+84.9)_n$	234	234	234

[a] Read pulse angles in degrees.

[b] All ϕ are ≤90° for suppression of off-resonance effects.

[c] Theoretical sensitivity enhancements relative to conventional quantitative $^{13}C\{^1H\}$ spectroscopy, for signals of carbon atoms with one (CH), two (CH_2), or three (CH_3) directly bonded protons.

3. RESULTS AND DISCUSSION

To confirm the ability of Q-DEPT spectroscopy for providing quantitative results, three spectra were collected using each of the four experiments in Table 1 for 45% ethylbenzene (C_6H_5-CH_2CH_3) in acetone-*d6*. Three spectra were also collected with conventional quantitative $^{13}C\{^1H\}$ experiments for comparison. Experiments were conducted at 150.897 MHz under conditions allowing complete signal relaxation and no cross-relaxation. The data sets, consisting of 65,536 complex points, 221 spectral windows, and 512 acquisitions, were multiplied by a 5 Hz line broadening factor before Fourier transformation into spectra and phase correction into pure absorption mode. Figure 3 illustrates representative spectra, where Q-DEPT spectra recorded with a 60.0° read pulse angle (Table 1, experiment 3) and a combination of 50.0, 35.6, 49.1, and 84.9° read pulse angles (Table 1, experiment 4) are shown together with a spectrum acquired under conventional quantitative $^{13}C\{^1H\}$ conditions. The increased sensitivity afforded by the Q-DEPT pulse sequences is immediately apparent by comparing the baseline noise of the Q-DEPT spectra (top and middle panels) to that of the conventional quantitative spectra (bottom panel). Also apparent when comparing the Q-DEPT and conventional spectra is the lack of the 29.8 ppm acetone-*d6* signal in the former case, resulting from the absence of a directly bonded ^1H nucleus to the acetone methyl carbon atoms preventing polarization transfer in the Q-DEPT acquisitions. As anticipated from Table 1, the differences in signal heights found between signals in the same spectrum can change when comparing one spectrum to another.

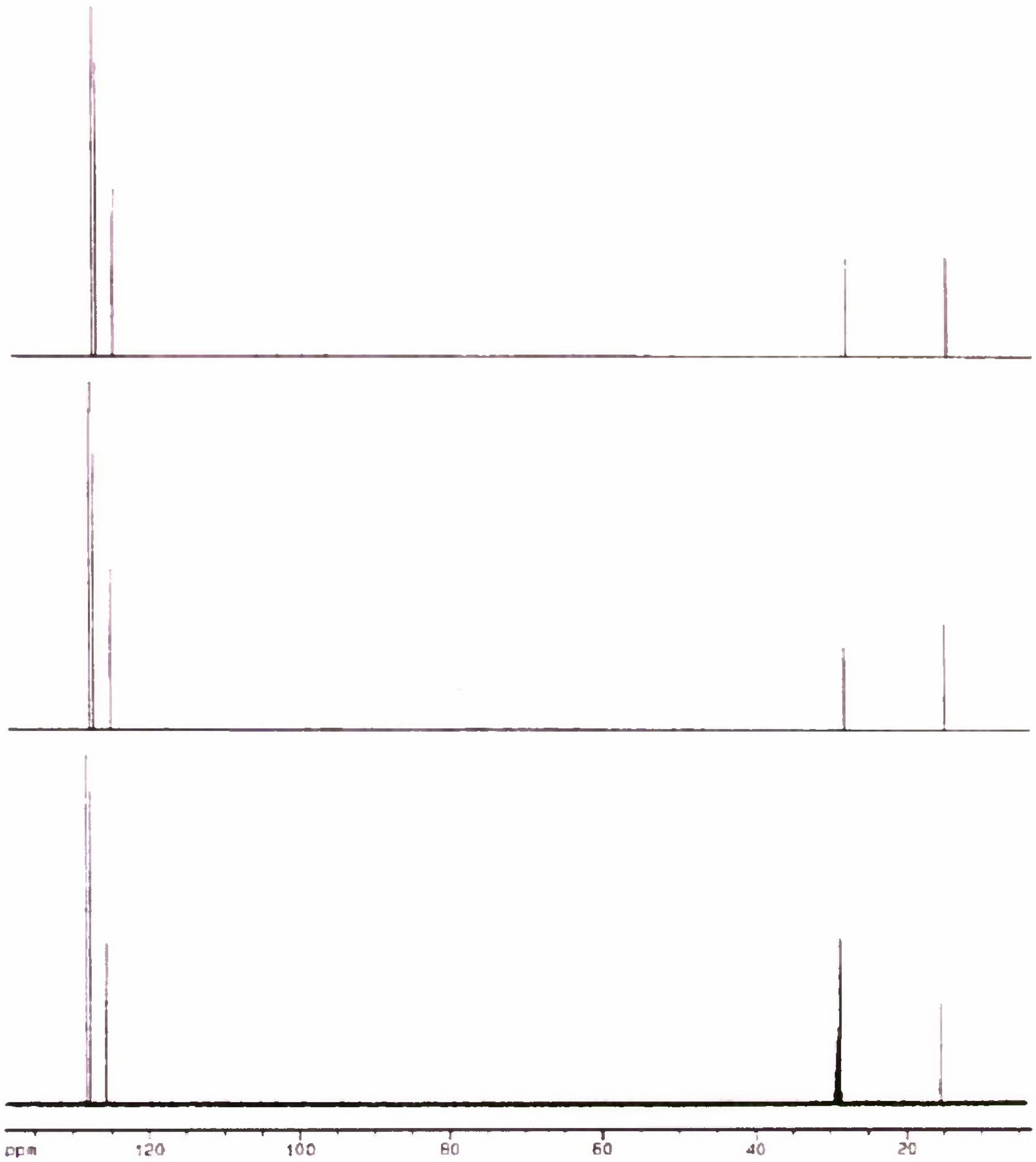

Figure 3. 150.897 MHz Q-DEPT Spectra of 45% Ethylbenzene in Acetone-*d6* Acquired with Read Pulse Angles of 60.0° (top) and a Combination of 50.0, 35.6, 49.1, and 84.9° (middle), Compared to a Corresponding Conventional Quantitative Spectrum (bottom). All spectra are from the summation of eight accumulations at 25° C, under identical conditions allowing complete signal relaxation and no cross-relaxation.

Table 2 summarizes the results of integrating the ethylbenzene signals. As anticipated, all signals (except those for aromatic ^{13}C from experiment 1 and methyl signals from experiment 3) have integral values very close to 1.00 when normalized to represent a single carbon atom. When these integrals are corrected for the intensity differences expected from their theoretical enhancements (Table 1), they have normalized values very close to 1.00 as well (not

shown). In addition, experiment 4 integrals are all similar to their corresponding integrals from the conventional experiments, which is a direct consequence of the θ cycling scheme equalizing the sensitivity enhancements of all protonated ^{13}C atoms. After adjusting the expected differences in sensitivity, little difference is evident between the spectra from all Q-DEPT and conventional quantitative spectroscopy.

Table 2. Quantitative Results for Ethylbenzene Using Q-DEPT and Conventional $^{13}C\{^1H\}$ Spectroscopy[a]

	Q-DEPT Experiment[b]				
Signal	1	2	3	4	$^{13}C\{^1H\}$[c]
CH_3	0.96 ± 0.01	1.05 ± 0.07	0.76 ± 0.01	0.95 ± 0.03	0.96 ± 0.05
p-CH	0.72 ± 0.01	0.91 ± 0.02	1.00 ± 0.07	1.09 ± 0.01	0.96 ± 0.04
m-CH	0.72 ± 0.02	0.91 ± 0.01	1.02 ± 0.04	1.09 ± 0.08	0.98 ± 0.09
o-CH	0.73 ± 0.03	0.92 ± 0.01	1.01 ± 0.04	1.08 ± 0.09	1.01 ± 0.05

a Table entries are from the integral values of ethylbenzene ^{13}C signals for the methyl group (CH_3) and the carbon atoms para (*p*-CH), meta (*m*-CH), and ortho (*o*-CH) to the ethyl group. Integrals are normalized to the methylene signal, assigned a value of 1.00 in all but experiment 2 spectra. Integrals of experiment 2 methylene signals were assigned a value of 284/246 or 1.15, derived from the sensitivity enhancements in Table 1. For simplicity, integral values were calculated to represent one carbon atom; i.e., *m*-CH signal integrals were divided by 2, and those of CH_3, by 1. Entries are reported ±95% confidence intervals.

b Experiments are described in Table 1.

c Data are from quantitative ^{13}C observation with inverse-gated ^1H decoupling.

4. CONCLUSIONS

Polarization transfer delays in the distortionless enhancement by polarization transfer (DEPT) pulse sequence have been modulated to create sensitivity-enhanced experiments for collecting quantitative $^{13}C\{^1H\}$ spectra. Four experiments, each with unique read pulse angles, give quantitative spectra with 200-300% more sensitivity than corresponding conventional methods [6]. The experiments can be used to acquire spectra with improved quantitative information or to substantially reduce the long acquisition durations indicative of quantitative ^{13}C experiments. While the ability of the experiments to provide quantitative spectra was confirmed by ethylbenzene, they can easily be adapted to analyze complex mixtures.

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